STUDIES ON NEWCASTLE DISEASE VIRUS

by

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INTRODUCTION

Newcastle disease is a virus disease of fowls, usually appearing in an acute form. The death rate is from 50 percent to 100 percent. It also affects egg production and egg quality (11). In general the disease causes a great loss to the poultry industry. The disease is widely distributed in America. It was very easily confused with fowl pest and it is now known that some of the outbreaks of the fowl plague in China considered as fowl pest were Newcastle disease (22). After the malady has become once established in a certain area, it is very likely to be prevalent every year but with seasonal variations. Many kinds of vaccines have been tried, but most of them are not satisfactory. Spreading of Newcastle disease is usually by contact. The digestive system and respiratory system are the common routes of infection. Secretions from the respiratory tract and fecal materials of diseased birds contain highly virulent virus, which may contaminate the soil. Soil is also very easily carried on shoes and tires from one place to another. Since very little is known concerning the resistance of the virus in the soil, a careful study was started on this subject in an attempt to help the control on Newcastle disease. An experiment on the resistance of the virus to direct sunlight was done, besides the soil experiments, in order to determine the resistance of the virus to sunlight.

The diagnosis of Newcastle disease is based on the isolation of the virus. Methods used to isolate and identify the virus are: (1) inoculating the material into birds, or chicken embryos; (2) hemagglutination and hemagglutination-inhibition tests. The last two tests are very commonly used in most laboratories. They are simple, quick, reliable and economical. Some relations between the living virus and its hemagglutinative ability are reported in this paper.

REVIEW OF LITERATURE

Newcastle disease was first reported in America by Beach as "A Respiratory Nervous Disorder". He named this disease pneumoencephalitis. Four years later, he found it to be identical with Newcastle disease (1). The causative agent was discovered by Doyle (13) to be a filtrable virus. According to the literature (3), Newcastle disease is widely distributed in the world. In America, it has been found in 42 states, including Kansas (8), and probably is present in others. Methods used to control this disease were outlined by U.S.D.A., B.A.I. (23). Bushnell and Twiehaus (9) also mentioned an important point "The runs must be plowed or spaded several times to expose the soil to the action of sunlight". But the nature of Newcastle disease virus is still little known to us. Some work has been contributed by others and may be summarized as follows:

In the soil. Farinas (14) found that spleen and liver from diseased birds covered with dry sandy earth for 12 days and crop content in the soil at room temperature for 4 days, failed to infect chickens.

To direct sunlight. Iyer (16) stated that the exposure of organ suspension to direct sunlight for one hour had no effect on the virus. But Farinas (14) reported that virus was killed by direct sunlight within a half hour and a 1:10 dilution of saliva in a cotton plugged tube was inactive after 24 hours exposure to daylight.

To heat. According to the work of Farinas (14), virus was inactivated within 30 minutes at 55 ^{o}C., but not at 50 ^{o}C. for the same time. Brandly, et al. (5) mentioned that strains E and 11914 were killed by heat at 60 ^{o}C. after 30 minutes, or within 45 ^{o}minutes at 55 ^{o}C. Iyer (16) said that the virus was killed by heating to 50^{o}C. or higher.

To pH. Moses, Brandly and Jones (20) reported that after a week's exposure, maximum stability was present within the range pH 5 to 9, and there was survival within the pH range of 2 to 11. These ranges were considerably broader than was demonstrable for fowl plague virus, which was more sensitive to an acid environment and had a maximum stability of pH 6 to 11. The stability patterns of fowl plague virus and its variant were essentially similar.

To ultraviolet light. Brandly et al. (5) exposed Newcastle disease virus under 2573 A wave length. They found Newcastle disease virus was inactivated in 35 to 55 minutes. Oppenheimer and Levinsion (21) produced inactivation of the Newcastle disease virus in 0.8 to 0.08 second, and of the fowl plague virus, in 1.1 to 3.0 seconds.

In the infected pen. From a summary of the work by Kaschula, Canham, Kiesel and Cole (cited by Blaxland), the Newcastle disease virus survived for less than 14 days in the infected pen (18).

At room temperature. According to the report of Iyer (17), the virus remained viable after storage for 21 days at room temperature (average 17° C.) but not after 28 days. Brandly et al. (5) found the activity of the amnio-allantoic fluid virus was retained at room temperature for 24 hours or longer.

To disinfectants. The results of Farinas (14) and Doyle (13) are summarized:

Agent	Virus inactive	Virus active
Potassium permangnate (13)	1:5000 in 1 hr.	1:10,000 in 1 hr.
Lysol (13)	1:1000 in 1 hr.	1:5000 in 1 hr.
Cresol (13)	1:500 in 1 hr.	1:1000 in 1 hr.
Carbolic acid (13)	1:20 in 1 hr.	1:100 in 1 hr.
Formalin (14)	1:50 in ½ hr.	1:100 in ½ hr.
Formalin (13)	1:50 in 1 hr.	
Methyl and ethyl alcohol (13)	1:2 in 1 hr.	1:4 in 1 hr.
Chloroform (14)	1:100 in ½ hr.	1:1000 in ½ hr.

Preservation of the virus. According to report of Doyle (13), the lung, liver, spleen, brain, and kidney kept in a cold, dry place, were positive at the fortieth day and negative at the hundredth day. But the ovary was nega-

tive both at fortieth day and the hundredth day. Virus in a 50 percent solution of glycerin in normal saline was active after 197 days, but was dead after 259 days. Virus was active up to 86 days in normal saline, but inactive at the hundredth day. The virus in a spleen kept in a Petri dish was active after 80 days; it was dead after 150 days. Blood in sealed pipettes was active after 109 days. According to the report of Beach (2), virus in lung, desiccated in vacuo over phosphorous pentoxide and stored in the refrigerator, remained viable for 195 days. In tissues, suspended in 50 percent glycerin and stored in the refrigerator, the virus was unchanged in 50 days; in 85 days its virulence had appreciably decreased. Virus in sealed ampoules stored in a "dry-ice" refrigerator has retained full virulence for 7 months, the longest period tested.

At 37° C. At 37° C., virus contained in liver and spleen pulp was active for 24 hours, where as the same strain of virus in buccal washings remained viable even after three days, according to Iyer (17).

The diagnosis of Newcastle disease is based on the isolation of the virus. The virus can be identified by the agglutination test with chicken red blood cells. The agglutination of chicken red blood cells by Newcastle disease virus was discovered by Burnet (7). The technic of making the test was modified by Lush (18) from the Hirst hemagglutination test with influenza virus. In 1946, the U.S.D.A. issued a publication about the hemagglutination test in the diagnosis of Newcastle disease which includes detailed directions for its use. Brandly et al. (6) discussed the technics of making the test in some detail and also the variations which are to be observed.

Chicken cells can be agglutinated by influenza virus, mumps virus and fowl plague virus, in addition to Newcastle disease virus. In the influenza virus, the relation between titers of hemagglutination and amount of virus has been established (10). Ginsberg et al. (15) strongly suggested that mumps

virus hemagglutination was caused by the virus particle itself and that the hemagglutination technique may indeed be used to measure virus concentration. When Cunha, Well, Beard, Taylor, Sharp and Beard (12) studied the purification and characteristics of the Newcastle disease virus, they found that the hemagglutinative activity of concentrated virus was proportional to infectivity on chicken embryos. In addition to the hemagglutination test for identifying the virus, the use of hemagglutination-inhibition test is considered a valuable confirmatory test in locating outbreaks of the disease. While the hemagglutination reaction is due to the presence of Newcastle disease virus; the hemagglutination-inhibition reaction depends on the presence of a Newcastle disease antibody.

EXPERIMENTAL METHODS AND RESULTS

Resistance of Newcastle Disease Virus in the Soil

Types of Soil. Three different kinds of soil were taken from the Kansas State College campus:

Soil P was taken at the surface of the ground under the pine trees near the Educational Hall; black in color; fine granular.

Soil W was taken from 2 feet under ground near the Illustration Building; clay-like soil; yellow in color; very fine granular.

Soil V was taken at the surface of the ground in front of Veterinary Hall; grey in color; larger granular.

Three hundred g of each soil was put into sterile jars with loose lids. Three samples were taken from each soil, one for control and the other two for inoculation with living Newcastle disease virus. One of the two inoculated soils was mixed thoroughly with 25 g of hydrated lime before inoculation. Jars were marked with numbers as follows:

Soil	Jar No.	Treatment	Final pH	Inoculated with virus	Remarks
P	1	No	6.2	Yes	———
P	2	Lime	11.18	Yes	———
P	3	No	6.2	No	Control
W	4	No	5.8	Yes	———
W	5	Lime	10.5	Yes	———
W	6	No	5.8	No	Control
V	7	No	5.6	Yes	———
V	8	Lime	10.2	Yes	———
V	9	No	5.2	No	Control

Nature and Source of Virus. The virus was provided by Dr. L.D. Bushnell. It was a Manhattan strain, harvested from chicken embryos. The highest hemagglutination titer was 1:2560 and the embryo M.L.D. was 1 times 10^{-9}. After

The virus was inoculated into the soils, about 2 g were taken from each jar every day, or every few days, mixed with 10 ml of sterile saline, filtered through Berkefeld filters, and a sterility test made. Two tenth ml. of each sterile filtrate was inoculated into three ten-day-old chicken embryos. These embryos were checked every day after inoculation. If the embryo was found dead, a sterility test was made, the sterile chorio-allantoic fluid was taken from the dead embryo and a hemagglutination test was run.

Technic of Making Hemagglutination Test. The techniques used for hemagglutination test followed exactly the publication of "Diagnosis of Newcastle Disease" from U.S.D.A. (24).

Experiment I. The amount of virus used was 5 ml of a 1:10 dilution, to each jar containing 300 grams fresh soil, or limed soil. Since the embryo M.L.D. of this virus was about 1:1,000,000,000 ml, each gram of soil would give about 17,000,000 embryo M.L.D. Embryos inoculated with 0.2 ml of filtrate from 10 ml saline mixture with 2.0 grams soil sample would receive 34,000 M.L.D., if the virus maintained its virulence. Results of the first experiment are given in Table 1.

Table 1. Survival of Newcastle disease virus in fresh soil.

A	Sa	Egg	C	Dilutions of chorio-all ntoic fluid used in HA test									Con-trol	Results
				1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280		
1	1	a	*7	+	-	-	-	-	-	-	-	-	-	V
1	1	b	*12	-	-	-	-	-	-	-	-	-	-	-
1	1	c												-
1	2	a	*12	-	-	-	-	-	-	-	-	-	-	-
1	2	b	*11	-	-	-	-	-	-	-	-	-	-	-
1	2	c												-
1	3	a	*9	-	-	-	-	-	-	-	-	-	-	-
1	3	b												-
1	3	c												-
1	4	a	*3	+	+	+	+	+	+	+	+	-	-	V
1	4	b												-
1	4	c												-
1	5	a	*9	-	-	-	-	-	-	-	-	-	-	-
1	5	b												-
1	5	c												-
1	6	a												-
1	6	b	*11	-	-	-	-	-	-	-	-	-	-	-
1	6	c												-
1	7	a	*2	+	+	+	+	+	+	+	+	-	-	V
1	7	b	*3	+	+	+	+	+	+	-	-	-	-	V
1	7	c	*11	-	-	-	-	-	-	-	-	-	-	-
1	8	a												-
1	8	b	*12	-	-	-	-	-	-	-	-	-	-	-
1	8	c												-
1	9	a												-
1	9	b												-
1	9	c												-
2	1	a	*3	+	+	+	+	+	+	+	+	+	-	V
2	1	b												-
2	1	c												-
2	2	a												-
2	2	b												-
2	2	c												-
2	3	a												-
2	3	b	*9	-	-	-	-	-	-	-	-	-	-	-
2	3	c												-
2	4	a												-
2	4	b												-
2	4	c												-
2	5	a												-
2	5	b												-
2	5	c	*8	-	-	-	-	-	-	-	-	-	-	-
2	6	a												-
2	6	b	*9	-	-	-	-	-	-	-	-	-	-	-
2	6	c												-
2	7	a												-
2	7	b	*4	+	+	+	+	+	+	+	+	+	-	V
2	7	c												-

Table 1. (cont.).

A	Sa	Egg	C	Dilutions of chorio-allantoic fluid used in HA test									Con-trol	Results
				1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280		
2	8	a												-
2	8	b												-
2	8	c												-
2	9	a												-
2	9	b												-
2	9	c												-
4	1	a												-
4	1	b												-
4	1	c												-
4	2	a	*1	-	-	-	-	-	-	-	-	-	-	-
4	2	b												-
4	2	c												-
4	4	a												-
4	4	b												-
4	4	c												-
4	5	a												-
4	5	b												-
4	5	c												-
4	7	a	*4	+	+	+	+	+	+	+	+	+	-	V
4	7	b	*5	+	+	+	+	+	+	+	+	-	-	V
4	7	c												-
4	8	a	*7	-	-	-	-	-	-	-	-	-	-	-
4	8	b												-
4	8	c												-
5	1	a	*1	-	-	-	-	-	-	-	-	-	-	-
5	1	b												-
5	1	c												-
5	4	a												-
5	4	b												-
5	4	c												-
5	7	a												-
5	7	b												-
5	7	c												-
6	1	a												-
6	1	b												-
6	1	c												-
6	4	a	*5	-	-	-	-	-	-	-	-	-	-	-
6	4	b												-
6	4	c												-
6	7	a												-
6	7	b												-
6	7	c												-

A.... time in days virus remained in soil.
Sa... sample taken from jar No. 1 to 9.
Egg.. eggs inoculated (eggs in triplicate).
C.... days to death of embryos.

*.... indicates a dead embryo
V.... living virus.
-.... negative result.
HA... hemagglutination

In the first experiment, the virus lived in fresh soil at room temperature without sunlight from 2 to 4 days, but in the soil treated with lime the virus lived less than one day.

Experiment II. Methods used were the same as in Experiment I, except that the amount of virus used was a 1:2 instead of a 1:10 dilution. Results are tabulated in Table 2. Samples of the first d y and the second from jar No. 1, 4 and 7 were omitted, because they gave positive result in Experiment I.

Table 2. Survival of Newcastle disease virus in fresh soil with heavier inoculation.

A	Sa	Egg	C	Dilutions of chorio-allantoic fluid used in HA test									Con-trol	Results
				1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280		
1	2	a												-
1	2	b												-
1	2	c												-
1	3	a												-
1	3	b	*b	-	-	-	-	-	-	-	-	-	-	-
1	3	c												-
1	5	a	*12	-	-	-	-	-	-	-	-	-	-	-
1	5	b	*12	-	-	-	-	-	-	-	-	-	-	-
1	5	c												-
1	6	a												-
1	6	b												-
1	6	c												-
1	8	a												-
1	8	b												-
1	8	c												-
1	9	a												-
1	9	b												-
1	9	c	*9	-	-	-	-	-	-	-	-	-	-	-
2	2	a												-
2	2	b												-
2	2	c												-
2	5	a												-
2	5	b	*5	-	-	-	-	-	-	-	-	-	-	-
2	5	c												-
2	8	a												-
2	8	b												-
2	8	c												-

Table 2. (cont.)

A	Sa	Egg	C	Dilutions of chorio-allantoic fluid used in HA test									Con-trol	Results
				1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280		
3	1	a	*3	+	+	+	+	+	+	+	+	+	-	V
3	1	b	*3	+	+	+	+	+	+	+	+	+	-	V
3	1	c	*3	+	+	+	+	+	+	+	+	+	-	V
3	4	a	*3	+	+	+	+	+	+	+	+	+	-	V
3	4	b												-
3	4	c												-
3	7	a	*3	+	+	+	+	+	+	+	+	+	-	V
3	7	b	*5	+	+	+	+	+	+	+	+	+	-	V
3	7	c	*5	+	+	+	+	+	+	+	+	+	-	V
4	1	a												-
4	1	b												-
4	1	c	*5	+	+	+	+	+	+	+	+	+	-	V
4	4	a	*5	+	+	+	+	+	+	+	+	+	-	V
4	4	b	*3	+	+	+	+	+	+	+	+	+	-	V
4	4	c												-
4	7	a												-
4	7	b												-
4	7	c												-
5	1	a												-
5	1	b												-
5	1	c												-
5	4	a												-
5	4	b	*5	-	-	-	-	+	+	+	+	+	-	V
5	4	c	*3	+	+	+	+	+	+	+	+	-	-	V
5	7	a												-
5	7	b	*3	+	+	+	+	+	+	+	+	+	-	V
5	7	c	*5	-	-	-	-	-	+	+	+	+	-	V
6	1	a												-
6	1	b	*12	-	-	-	-	-	-	-	-	-	-	-
6	1	c												-
6	4	a	*2	-	-	-	-	-	-	-	-	-	-	-
6	4	b												-
6	4	c	*5	+	+	+	+	+	+	+	+	-	-	V
6	7	a												-
6	7	b												-
6	7	c	*3	-	-	-	-	-	-	-	-	-	-	-
8	1	a	*12	-	-	-	-	-	-	-	-	-	-	-
8	1	b												-
8	1	c	*12	-	-	-	-	-	-	-	-	-	-	-
8	4	a												-
8	4	b	*2	-	+	+	-	-	-	-	+	+	-	V
8	4	c	*2	+	+	+	+	+	+	+	+	+	-	V

Table 2. (cont.)

A	Sa	Egg	C	Dilutions of chorio-allantoic fluid used in HA test									Con-trol	Results
				1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280		
8	7	a												-
8	7	b												-
8	7	c												-
9	4	a	*12	-	-	-	-	-	-	-	-	-	-	-
9	4	b	*12	-	-	-	-	-	-	-	-	-	-	-
9	4	c												-
10	4	a	*5	-	-	-	-	-	-	-	-	-	-	-
10	4	b												-
10	4	c												-

See footnote Table 1.

From the second experiment, it was found that with a heavy inoculation, the virus could live from two to eight day. However, in the soil treated with lime (25 g lime in 300 g soil), the virus died within 24 hours.

Experiment III. Experiments I and II were repeated; fresh soils and virus used were the same as in Experiments I and II. The amount of lime used was decreased to five g for soil No. 2, 5, 8, 2', 5' and 8'. Table 3 presents the results of this experiment where soil No. 1', 2', 4', 5', 7' and 8' were inoculated with 5.0 ml virus in 1:2 dilution.

Table 3. Survival of Newcastle disease virus in fresh soil with light and heavy inoculation.

A	Sa	Egg	C	Dilutions of chorio-allantoic fluid used in HA test									Con-trol	Results
				1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280		
1	2	a												-
1	2	b	*3	+	+	+	+	+	-	-	-	-	-	V
1	2	c												-
1	2'	a												-
1	2'	b												-
1	2'	c	*8	-	-	-	-	-	-	-	-	-	-	-
1	5	a												-
1	5	b	*2	-	-	-	-	-	-	-	-	-	-	-
1	5	c	*2	+	+	+	+	+	+	+	+	-	-	V
1	5'	a												-
1	5'	b	*5	+	+	+	+	-	-	-	-	-	-	V
1	5'	c												-
1	8	a	*9	-	-	-	-	-	-	-	-	-	-	-
1	8	b												-
1	8	c												-
1	8'	a												-
1	8'	b												-
1	8'	c												-
1	3	a												-
1	3	b												-
1	3	c	*10	-	-	-	-	-	-	-	-	-	-	-
1	6	a												-
1	6	b												-
1	6	c												-
1	9	a	*4	-	-	-	-	-	-	-	-	-	-	-
1	9	b												-
1	9	c												-
2	2	a	*12	-	-	-	-	-	-	-	-	-	-	-
2	2	b	*12	-	-	-	-	-	-	-	-	-	-	-
2	2	c	*12	+	+	+	+	+	-	-	-	-	-	V

Table 3. (cont.).

A	Sa	Egg	C	Dilutions of chorio-allantoic fluid used in HA test 1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	Control	Results
2	5	a												-
2	5	b	*6	-	-	-	-	-	-	-	-	-	-	-
2	5	c												-
2	8	a	*8	-	-	-	-	-	-	-	-	-	-	-
2	8	b												-
2	8	c												-
2	2'	a	*9	-	-	-	-	-	-	-	-	-	-	-
2	2'	b	*8	-	-	-	-	-	-	-	-	-	-	-
2	2'	c												-
2	5'	a	*12	-	-	-	-	-	-	-	-	-	-	-
2	5'	b												-
2	5'	c												-
2	8'	a	*8	-	-	-	-	-	-	-	-	-	-	-
2	8'	b												-
2	8'	c												-
3	2	a	*9	-	-	-	-	-	-	-	-	-	-	-
3	2	b	*9	-	-	-	-	-	-	-	-	-	-	-
3	2	c	*6	-	-	-	-	-	-	-	-	-	-	-
3	2'	a												-
3	2'	b												-
3	2'	c												-
3	5	a	*7	-	-	-	-	-	-	-	-	-	-	-
3	5	b	*7	-	-	-	-	-	-	-	-	-	-	-
3	5	c	*7	-	-	-	-	-	-	-	±	-	-	-
3	5'	a	*5	-	-	-	-	-	-	-	-	-	-	-
3	5'	b	*6	-	+	+	+	+	+	+	-	-	-	V
3	5'	c	*9	-	-	-	-	-	-	-	-	-	-	-
3	8	a	*9	-	-	-	-	-	-	-	-	-	-	-
3	8	b	*10	+	-	-	-	-	-	-	-	-	-	V
3	8	c	*8	-	-	-	-	-	-	-	-	-	-	-
3	8'	a	*6	-	-	-	-	-	-	-	-	-	-	-
3	8'	b	*8	-	-	-	+	+	+	+	+	-	-	V
3	8'	c	*7	-	-	-	-	-	-	-	-	-	-	-
3	1	a	*12	-	-	-	-	-	-	-	-	-	-	-
3	1	b												-
3	1	c												-
3	1'	a	*3	-	-	-	-	-	-	-	-	-	-	-
3	1'	b												-
3	1'	c												-
3	7	a	*9	-	-	-	-	+	+	+	+	+	-	V
3	7	b	*8	-	-	-	-	-	-	-	-	-	-	-
3	7	c												-
3	7'	a	*3	+	+	+	+	+	+	-	-	-	-	V
3	7'	b	*3	+	+	+	+	+	+	+	+	-	-	V
3	7'	c	*12	-	-	-	-	-	-	-	-	-	-	-
3	4	a	*9	+	+	+	+	+	+	-	-	-	-	V
3	4	b	*3	+	+	+	+	+	+	+	+	+	-	V
3	4	c	*5	+	+	+	+	+	+	+	-	-	-	V

Table 3. (cont.)

A	Sa	Egg	C	Dilutions of chorio-allantoic fluid used in HA test 1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	Control	Results
3	4'	a	*8	–	+	+	+	–	–	–	–	–	–	V
3	4'	b	*5	+	+	+	+	+	+	+	+	–	–	V
3	4'	c												–
4	1	a												–
4	1	b												–
4	1	c												–
4	1'	a	*8	–	–	–	–	–	–	–	–	–	–	–
4	1'	b	*12	–	–	–	–	–	–	–	–	–	–	–
4	1'	c	*12	–	–	–	–	–	–	–	–	–	–	–
4	4	a												–
4	4	b	*10	–	–	–	–	–	–	–	–	–	–	–
4	4	c												–
4	4'	a	*9	–	+	+	+	+	+	+	+	+	–	V
4	4'	b	*9	–	–	–	–	+	+	+	+	–	–	V
4	4'	c	*3	+	+	+	+	+	+	+	+	+	–	V
4	7	a	*3	+	+	+	+	+	+	–	–	–	–	V
4	7	b	*9	+	+	+	+	–	–	–	–	–	–	V
4	7	c	*9	–	–	–	–	–	–	–	–	–	–	–
4	7'	a												–
4	7'	b	*9	+	+	+	+	+	+	+	+	–	–	V
4	7'	c												–
5	1'	a	*9	+	+	–	–	–	–	–	–	–	–	V
5	1'	b	*9	–	–	–	–	–	–	–	–	–	–	–
5	1'	c												–
5	1	a	*5	±	–	–	–	–	–	–	–	–	–	–
5	1	b	*9	–	–	–	–	–	–	–	–	–	–	–
5	1	c												–
5	4'	a	*10	–	–	–	–	–	–	–	–	–	–	–
5	4'	b												–
5	4'	c	*10	–	–	–	–	–	–	–	–	–	–	–
5	4	a												–
5	4	b												–
5	4	c												–
5	7'	a												–
5	7'	b												–
5	7'	c												–
5	7	a												–
5	7	b												–
5	7	c												–
6	1'	a												–
6	1'	b	*6	–	–	–	–	–	–	+	+	+	–	V
6	1'	c												–
6	1	a												–
6	1	b												–
6	1	c												–
6	4'	a	*6	–	–	–	+	+	+	+	+	+	–	V
6	4'	b	*6	–	–	–	+	–	+	+	+	+	–	V
6	4'	c												–

Table 3. (cont.).

A	Sa	Egg	C	Dilutions of chorio-allantoic fluid used in HA test 1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	Control	Results
6	4	a	*5	-	-	-	-	-	-	-	-	-	-	-
6	4	b	*8	-	-	-	-	-	-	-	-	-	-	-
6	4	c	*6	-	-	-	-	+	-	-	-	-	-	-
6	7'	a	*6	-	-	-	-	-	-	+	+	+	-	V
6	7'	b	*7	-	-	-	-	-	-	-	-	-	-	-
6	7'	c												-
6	7	a	*7	-	-	-	-	-	-	-	-	-	-	-
6	7	b	*7	-	-	-	-	-	-	-	-	-	-	-
6	7	c	*7	-	-	-	-	-	-	-	-	-	-	-
7	1'	a	*8	+	+	+	+	+	-	-	-	-	-	V
7	1'	b												-
7	1'	c												-
7	4'	a												-
7	4'	b												-
7	4'	c	*9	-	-	-	-	-	-	-	-	-	-	-
7	7'	a												-
7	7'	b	*9	-	-	-	-	-	-	-	-	-	-	-
7	7'	c	*7	-	-	-	-	-	-	-	-	-	-	-
9	1'	a												-
9	1'	b												-
9	1'	c												-
9	4'	a												-
9	4'	b	*7	-	-	-	-	-	-	-	-	-	-	-
9	4'	c												-
9	7'	a	*9	-	-	-	-	-	-	-	-	-	-	-
9	7'	b	*7	-	-	-	+	+	+	+	+	+	-	V
9	7'	c	*6	-	-	-	-	-	-	-	-	-	-	-
10	1'	a												-
10	1'	b	*7	-	-	-	-	-	-	-	-	-	-	-
10	1'	c												-
10	4'	a												-
10	4'	b	*8	-	-	-	-	-	-	-	-	-	-	-
10	4'	c	*8	-	-	-	-	-	-	-	-	-	-	-
10	7'	a												-
10	7'	b												-
10	7'	c												-
11	1'	a	*7	-	-	-	-	-	-	-	-	-	-	-
11	1'	b	*7	-	-	-	-	-	-	-	-	-	-	-
11	1'	c	*8	-	-	-	-	-	-	-	-	-	-	-
11	4'	a	*6	-	-	-	-	-	-	-	-	-	-	-
11	4'	b	*6	-	-	-	-	-	-	-	-	-	-	-
11	4'	c	*8	-	-	-	-	-	-	-	-	-	-	-
11	7'	a												-
11	7'	b												-
11	7'	c	*6	-	-	-	-	-	-	-	-	-	-	-

See footnote Table 1.

A few embryos died from bacterial contamination, but the numbers were insignificant. Some embryos died not from virus or from bacterial contamination, probably from the ineffectiveness of the incubator and other reasons. While a few embryos died the twelfth day following inoculation, they contained little chorio-allantoic fluid. In such cases, half ml sterile saline was put into the dead embryo and mixed with it. Then this half ml was taken out from the embryo and hemagglutination test was made on it.

Results and Discussions of Experiments I, II and III. When 300 g of fresh soil were inoculated with 5.0 ml virus of 1:10 dilution, the virus lived in soil from two to four days. Results obtained from Experiments I and III were the same.

When 300 g of fresh soil were inoculated with 5.0 ml virus of 1:2 dilution, the virus lived in the soil for 8 days in Experiment II and 9 days in Experiment III. Those two experiments gave close correlation.

Both in Experiment I and in Experiment II, when 300 g of fresh soil were mixed with 25 g of lime the virus lived for less than 24 hours.

When 300 g of fresh soil were mixed with 5.0 g of lime, the virus lived in the light-limed soil for 3 days, or more.

The surviability of Newcastle disease virus in the soil was related to the concentration of the virus. When virus used was a 1:2 dilution, it could survive up to 9 days, while in a 1:10 dilution for only four days.

Samples were taken from the soils and inoculated into embryos in triplicate. When three embryos remained alive in a single inoculation, it did not mean that there was no virus present. For this reason, additional samples were taken and inoculations continued for two additional days. If no further

positive results were obtained testing was discontinued. Irregular results were encountered in these experiments as, for example, when the sample of the first day was negative, and the following day positive.

Example:

Days	Sample	Results	Days	Sample	Results
4	7	negative	5	7	positive in Experiment II
2	5'	negative	3	5'	positive in Experiment III
2	8'	negative	3	8'	positive in Experiment III
4	1'	negative	5	1'	positive in Experiment III

This condition may be due to two reasons. First, three living embryos may not be enough to detect virus in the sample when the concentration is low. Second, virus particles may not be evenly distributed throughout the soil.

Survival of Newcastle Disease Virus at 37° C.

Ten ml of chorio-allantoic fluid virus were diluted ten times with sterile saline. Five ml of this dilution were put into sterile test tubes with screw caps. One tube was taken from the incubator every day and 0.2 ml was inoculated into three living chicken embryos as in Experiment I. Hemagglutination tests were made on those embryos which died. The results are tabulated as follows:

Table 4. Survival of Newcastle disease virus at 37° C.

Days at 37 C.	Egg No. inoc.	C	Dilutions of chorio-allantoic fluid used in HA test									Con-trol	Results
			1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280		
1	a	*2	+	+	+	+	+	+	+	+	+	-	V
	b	*2	+	+	+	+	+	+	+	+	+	-	V
	c	*2	+	+	+	+	+	+	+	+	+	-	V
2	a	*7	-	-	-	-	+	+	+	+	+	-	V
	b												-
	c												-
3	a	*5	-	-	-	-	-	+	+	+	+	-	V
	b	*5	-	-	+	-	-	+	+	+	+	-	V
	c												-
4	a												-
	b												-
	c												-
5	a	*4	-	-	-	-	-	-	-	-	-	-	-
	b	*12	-	-	-	-	-	-	-	-	-	-	-
	c	*6	+	+	+	+	+	+	-	-	-	-	V
7	a	*12	-	-	-	-	-	-	-	-	-	-	-
	b												-
	c												-
8	a	*7	-	-	-	-	-	-	-	-	-	-	-
	b	*7	-	-	+	+	+	+	+	+	-	-	V
	c												-
9	a												-
	b												-
	c												-
10	a												-
	b	*9	-	-	-	-	-	-	-	-	-	-	-
	c												-
11	a												-
	b												-
	c												-
12	a	*11	-	-	-	-	-	-	-	-	-	-	-
	b												-
	c												-

See footnote Table 1.

After the sample was taken from the 37° C. incubator, in addition to inoculation of the three chicken embryos, hemagglutination tests were also made. The changes of hemagglutinative titer in different samples were as follows:

Table 5 Changes of hemagglutinative titer of the original samples of Newcastle disease virus at 37° C.

Days at 37° C.	Dilutions of the sample for hemagglutination test									Control	Egg killed in 3-inoc.
	1:5	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280		
1	+	+	+	+	+	+	+	+	+	-	3/3*
2	+	+	+	+	-	-	-	-	-	-	1/3
3	+	+	+	+	-	-	-	-	-	-	2/3
4	+	+	+	-	-	-	-	-	-	-	0/3
5	+	+	+	-	-	-	-	-	-	-	1/3
6											
7	+	+	+	-	-	-	-	-	-	-	0/3
8	+	+	±	±	-	-	-	-	-	-	1/3
9	+	+	-	-	-	-	-	-	-	-	0/3
10	+	+	-	-	-	-	-	-	-	-	0/3
11	-	-	-	-	-	-	-	-	-	-	0/3
12	-	-	-	-	-	-	-	-	-	-	
13	-	-	-	-	-	-	-	-	-	-	
14	-	-	-	-	-	-	-	-	-	-	

*Numerator.......... number died
Denominator........ number inoculated

From this single experiment it may be concluded that the virus retained enough virulence to infect chicken embryos for 8 days at 37° C. But the virulence dropped very quickly during the second day. Since only three eggs were used each time, and this was just a single experiment, no definite curve could be established to illustrate the decrease of virulence. However, the hemagglutinative titer dropped regularly as illustrated in Fig. 1.

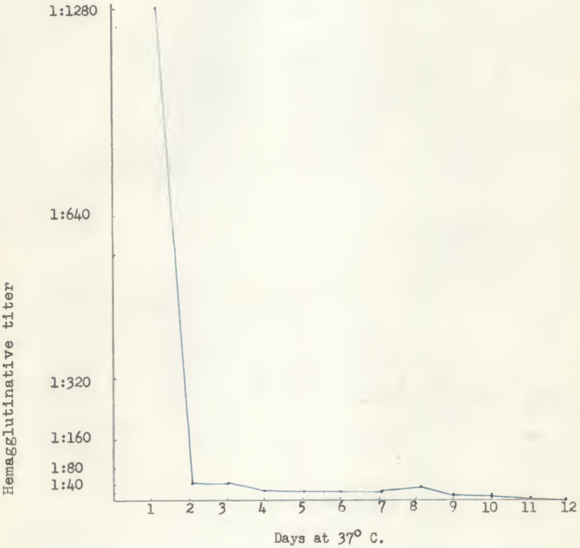

Fig. 1. Hemagglutinative titer of virus held at 37° C.

Effect of Direct Sunlight on Newcastle Disease Virus

Cotton cloth was cut into small pieces about 0.5 by 1.5 inches in size. These were sterilized in an autoclave and then saturated with allantoic virus. The virus used was the same as in Experiment I. In one group, each single piece of cloth was spread in a sterile Petri dish without a cover. In a second group, pieces of cloth were overlapping and put in another sterile Petri dish also without cover. Petri dishes were exposured to direct sunlight out of doors. The date for this experiment was June 5th, 1948 with good strong summer sunlight. The time was from two to five o'clock. From the first group, a piece of cloth was taken from the direct sunlight in 30 minutes, one hour and three hours, respectively. From the second group, a piece of cloth was taken from under two layers of cloth, after three hours exposure to sunlight. Each piece of cloth which received sunlight directly or indirectly was soaked in a small test tube with three ml of sterile saline for 10 minutes at room temperature and was shaken frequently. After 10 minutes, the three ml of saline suspension was filtered with a small Seitz filter in a centrifuge. A sterility test was made after filtration. Three ten-day-old living chicken embryos were inoculated with 0.2 ml from each filtrate. Inoculated embryos were checked each day and hemagglutination test made on the dead embryos as was done in Experiment I. It was found that the virus was inactivated by direct sunlight within 30 minutes. But virus on the piece of cloth which was protected by two layers of cloth was still living after three hours.

Distribution of the Death of Embryos After the Inoculation with Materials Containing Newcastle Disease Virus

Samples were collected from the soil, hay, straw, cloth and rubber after they had been contaminated with Newcastle disease virus. Virus used was the same as in Experiment I. Death date of embryos killed by the inoculation with virus is summarized in Table 6.

Table 6. Distribution of the time of the death of embryos after inoculation with materials containing Newcastle disease virus**.

Death day of embryos	Numbers of embryos which died	Percentage of dead embryos
1	0	0
2	6	8.69
3	23	33.30
4	3	4.30
5	7	10.13
6	11	15.99
7	1	1.45
8	6	8.69
9	9	13.11
10	2	2.89
11	0	0
12	1	1.45

** The data included in Table 6 was obtained from various experiments and summarized in this form.

The greatest number of embryos died on the third day after inoculation of samples which contained virus. These results coincided with those reported by Beaudette (3). The results of our observations, and those of Beaudette, are shown graphically in Fig. 2. But in his results about 90 percent of the embryos were killed by virus before the third day after inoculation while there were only 42.26 percent for the same time according to these data. The probable reason for this was that the material which he used was concentrated virus. The data recorded in this table were from different samples containing various amounts of virus. From the data in Table 6 it is obvious that embryos inoculated with materials containing Newcastle disease virus should not be dis-

carded until the embryo is dead or has hatched. A few embryos contains virus died as late as the 12th day after inoculation. Most investigators discard living embryos after six days of incubation. This is too early to obtain complete data.

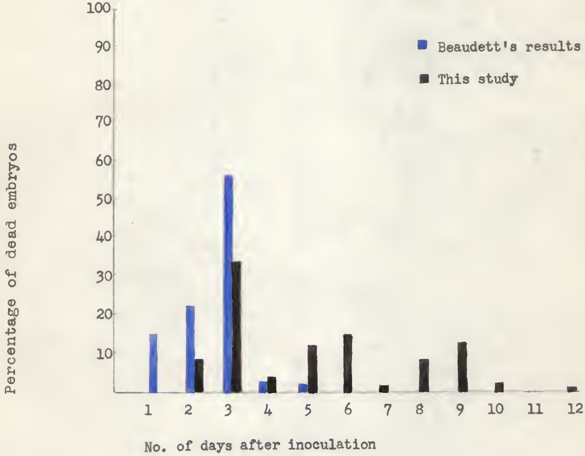

Fig. 2 Distribution of the number of dead embryos after the inoculation with material containing Newcastle disease virus.

Relation Between the Hemagglutinative Titer and the Virulence of the Newcastle Disease Virus

Sixty-nine samples were collected from the soil, hay, straw, cloth and rubber after contamination with Newcastle disease virus and were soaked in saline. The hemagglutinative titer of the 69 saline extract was determined before embryos were inoculated. The following table gives the results.

Table 7 Relation between the hemagglutinative titer and the virulence of the Newcastle disease virus.

Hemagglutinative titer of the sample	Embryos killed by the virus in the sample	
	Numbers of embryos	Percentage
1:1280	3/3**	100
1:160	1/3	33
1:40	7/18	38
1:20	8/18	44
1:10	2/24	8
1:5	3/21	25
1:1	0/9	0

** Numerator........... number died
Denominator......... number inoculated

From the above data, generally speaking, materials with higher hemagglutinative titer killed a larger percentage of embryos. When the hemagglutinative titer was below 1:20 three embryos were not enough to detect virus in the sample, because the infectivity was less than 33 percent. When the hemagglutinative titer of such materials falls as low as 1:1, it may or may not contain living virus.

In another group, data were obtained on 95 samples. Thirteen showed no hemagglutinative titer, but embryos were actually killed by the Newcastle disease virus. From this it may be said that the hemagglutination test may be used to detect the amount of Newcastle disease virus when the concentration of virus is high.

Relation Between the Hemagglutinative Titer of the Chorio-allantoic Fluid from the Dead Embryos and the Elapse Time Between Inoculation and Death

The hemagglutinative titer of each material inoculated into embryos was determined before inoculation. Embryos died on different dates and the hemagglutinative titer of chorio-allantoic fluid was determined. The relation between the hemagglutinative titer of the chorio-allantoic fluid from the dead embryos and the period between inoculation and death is shown in Table 8.

Table 8. Relation between the hemagglutinative titer of the chorio-allantoic fluid from the dead embryos and the date of death of the embryos.

Time embryos killed by virus in days	Mean of the hemagglutinative titer of the chorio-allantoic fluid from the dead embryos	Mean of the hemagglutinative titer of original sample	Number of embryos killed
2	1:530	1:120	6
3	1:940	1:40	23
4	1:980	1:160	3
5	1:960	1:8.60	7
6	1:740	1:0.70	11
8	1:383	1:4.50	6
9	1:1.45	1:3.10	9
10	1:5	1:5	2
12	1:80	1:2	1

From these data, it may be observed that embryos dead before the sixth day after inoculation always gave higher hemagglutinative titer in their chorio-allantoic fluid than those living longer, while embryos dying after the dixth day, may or may not give a good agglutinative titer.

SUMMARY

1. Depending upon the amount of virus in the soil, Newcastle disease virus lived in the fresh soil from two to nine days.

2. When Newcastle disease virus was introduced into 300 g of fresh soil which had been mixed with 25 g of lime, the virus was inactivated within 24 hours.

3. The virus was inactivated by direct sunlight within 30 minutes. When the virus was covered with 2 layers of cloth it was still living after three hours' exposure to sunlight.

4. The Newcastle disease virus retained its infectivity to chicken embryos for 8 days at 37° C. From day to day at 37° C., both the hemagglutinative titer and the infectivity of the virus decreased rapidly.

5. There was a close relation between hemagglutinative titer of the sample and the infectivity of the sample when the hemagglutinative titer of the sample was above 1:20.

6. More embryos died on the third day after inoculation with virus than for any other period. Over 70 percent of the embryos died before the sixth day after inoculation. Some embryos were killed by the virus as late as 12 days following inoculation.

7. Relatively high hemagglutinative titers were obtained from the chorio-allantoic fluid of embryos which died before the sixth day after inoculation.

8. Hemagglutination test was useful in detecting the amount of Newcastle disease virus when the sample had a high concentrations. It was of no use when the sample had a low concentration.

CONCLUSIONS

Newcastle disease virus lived in fresh soil from two to nine days. It is possible that soil is an important factor in spreading the disease within a flock, or from one flock to another by soil carried on shoes and tires. In this experiment, 0.04 g or less of contaminated soil was enough to kill a living embryo.

The viability of Newcastle disease virus in the soil was directly related to its original concentration. Early diagnosis in a flock is helpful in getting rid of the disease. When more birds die in a flock there will be more virus in the soil by contamination from respiratory secretion and fecal material.

When 300 g of soil were treated with 25 g of lime, the virus was inactivated within 24 hours. Direct sunlight killed the virus within 30 minutes. For this reason, soil contaminated with virus should be plowed or spaded several times to expose the soil to the action of sunlight as mentioned by Dr. Bushnell, or mixed with lime, or both.

In detecting Newcastle disease virus, inoculated embryos should be checked twice every day. If embryos are found dead they should be removed from the incubator immediately. It has been found that after 24 hours, the infectivity and hemagglutinative titer of the virus in dead embryo decreased rapidly and tests with such material may give false negative results. A few embryos inoculated with a material containing a low concentration of Newcastle disease virus may die of virus infection as late as 12 days after inoculation. From this it is evident that inoculated embryos should not be discarded until they have hatched or died.

In order to get virus with a very high hemagglutinative titer from dead embryos, the allantoic fluid should be collected as soon as possible after death. Embryos which die after the sixth day may give a comparatively low hemagglutinative titer.

It can be concluded that the hemagglutination test is most useful for detecting the amount of Newcastle disease virus in a sample when the concentration of virus is high. When the hemagglutination titer of a sample is below 1:20, triplicate inoculations into chicken embryos may fail in detecting the virus because the infectivity is below 33 percent.

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